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Genome-wide association study to identify variants associated with vaso-occlusive pain in sickle cell anemia

Shruti Chaturvedi[1], **Pallav Bhatnagar**[2], **Christopher J. Bean**[3], **Martin H. Steinberg**[4], **Jaqueline N. Milton**[5], **James F. Casella**[6], **Emily Barron-Casella**[6], **Dan E. Arking**[2], and **Michael R. DeBaun**[7]

[1]Division of Hematology and Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

[2]McKusick-Nathans Institute of Genetic Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD

[3]Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA

[4]Departments of Medicine, Pediatrics, Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA

[5]Department of Biostatistics, Boston University School of Public Health, Boston, MA

[6]Department of Pediatrics, Division of Pediatric Hematology, School of Medicine, Johns Hopkins University, Baltimore, MD

[7]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN

To the editor

Acute vaso-occlusive pain episodes are a hallmark of sickle cell anemia (SCA), one of the most common Mendelian disorders worldwide with an estimated >300,000 births annually.[1–3] Although SCA is a monogenic disorder, manifestations and disease severity are highly variable, suggesting additional phenotypic modifiers. The few genetic factors known to act as phenotypic modifiers do not completely explain the clinical heterogeneity in SCA. Previous genetic association studies identified that variants at 3 distinct loci (*BCL11A*, *HBS1L-MYB*, and *HBB*) are strong determinants of fetal hemoglobin level, and single nucleotide polymorphism (SNP) variant rs6141803 located upstream of *COMMD7* is associated with acute chest syndrome.[4] Also, heme-oxygenase-1 gene (*HMOX-1*) promoter polymorphisms influence heme oxygenase (HO-1) activity and incidence of acute chest

Address correspondence to: Michael R. DeBaun, MD, MPH, Division of Hematology-Oncology, Vanderbilt-Meharry Center in Sickle Cell Disease, 2200 Children's Way, Ste 11101, Nashville, TN 37232, Phone: (615) 875-3040, Fax: (615) 875-3055, m.debaun@vanderbilt.edu.

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syndrome in children with sickle cell disease (SCD).[5,6] We conducted this genome wide association study (GWAS) to identify variants associated with acute severe vaso-occlusive pain in children with SCA enrolled in the Cooperative Study for Sickle Cell Disease (CSSCD) and Silent Infarct Transfusion (SIT) trial. The CSSCD, a multi-institutional prospective cohort natural history study of SCD, enrolled 3,538 individuals with SCD between 1979 and 1981.[7] The SIT Trial, a multi-center international trial, screened 1,210 children with SCA to test the hypothesis that regular blood transfusions attenuate progression of cerebral infarcts in children with pre-existing silent strokes.[8] Both studies were approved by the Institutional Review Boards at Boston University School of Medicine and Vanderbilt University Medical Center.

We included participants identified as being of African descent from both cohorts, with available genotype data, and diagnosed with SCA. We excluded participants if essential clinical or demographic data (necessary for phenotypic assignment or previously reported to impact the pain phenotype) was missing, or if there was discordance between genetically defined and self-identified sex. We excluded all self-reported first-degree relatives, and cryptic relatedness (including full siblings, parents, offspring) determined by examining pairwise identity-by-descent in the combined cohort. To harmonize pain phenotypes in the CSSCD and SIT Trial cohorts, age inclusion criteria of 2 to 18 years was used to match the age and length of follow-up in both cohorts. SIT trial participants were between 5 and 15 years of age at the time of registration and included a 3-year retrospective collection of all acute severe vaso-occlusive pain based on hospitalization and treatment with opioid medication.[10] Unlike previous CSSCD pain analyses, where the definition of a pain episode included an acute vaso-occlusive event that lasted at least two hours and resulted in a physician visit,[9] we restricted the definition of a pain episode to include only episodes requiring hospitalization, to match the SIT trial definition.

CSSCD cohort DNA samples were genotyped at Boston University School of Medicine using Illumina Human610-Quad arrays (n=610,000 SNPs) (Illumina, San Diego, CA, USA) and BeadStudio was used to call genotypes. SIT trial samples were genotyped at Center for Inherited Disease Research (CIDR) at Johns Hopkins University School of Medicine (N=573) using the Illumina HumanHap650Y array (n=661,000 SNPs) (Illumina Inc., San Diego CA, USA) or at the Center for Disease Control, Atlanta, GA, USA (N=509) using the Illumina Infinium HumanOmni1-Quad array (n=1,134,514 SNPs) (Illumina Inc., San Diego CA, USA). After detailed quality control (QC) procedures (and excluding CSSCD samples outside the SIT Trial age inclusion criteria (≥2 and ≤12 years), 359 and 934 samples from the CSSCD and SIT cohorts, respectively, were included in the analysis (Table 1, Supplemental Figures S1A and S1B, Supplemental methods). To infer un-genotyped SNPs and fill in missing data across genotyping platforms in the SIT trial and CSSCD cohort, we merged HumanHap650Y, HumanOmni1-Quad and Human610-Quad array datasets and performed imputation for autosomal markers using a Hidden Markov model as implemented in MaCH, version 1.16,[10] with 50 rounds and 200 states. QC was performed both before and after imputation and poorly imputed SNPs (Rsq <0.3, squared correlation between imputed and true genotypes) were excluded; a total of 1,098,907 SNPs remained for analysis. Due to the observed over-dispersion of pain episodes in both cohorts, a multivariate quasi-Poisson regression model with correction for estimated over-dispersion, was used to evaluate

possible associations between SNPs and acute vaso-occlusive pain rate, treated as a quantitative trait. The model was adjusted for age at enrollment, sex, hematocrit, and the top 10 principal components from the genetic data (to account for population substructure and genetic heterogeneity), assuming additive effects of allele dosage on acute vaso-occlusive pain rate.

Participant characteristics for the SIT trial and CSSCD cohort are shown in Table 1. Statistically significant, but not clinically relevant, differences were identified between the two cohorts in age, percentage of fetal hemoglobin, reticulocyte percentage, pain rate, and follow-up time. The Manhattan plot summarizing the results of GWAS for acute vaso-occlusive pain in the SIT trial and CSSCD cohort for the additive model is shown in Figure 1. The genomic inflation lambda coefficient was 1.079, suggesting minimal test statistic inflation by potential population stratification, cryptic relatedness, or other technical factors. While none of the SNPs were significant at $P < 5.0 \times 10^{-8}$, one novel locus approached genome-wide significance: SNP rs3115229 ($P = 5.63 \times 10^{-8}$). This SNP is located 63.7 kb 5′ upstream of the *KIAA1109* gene on chromosome 4 (4q27).

The suggested locus includes the *KIAA1109-TENR-IL2-IL21* linkage disequilibrium block, containing three known protein-coding genes, *TENR*, *IL2*, *IL21,* and a predicted gene of unknown function, *KIAA1109*. This locus has been associated with auto-inflammatory disorders such as celiac disease,[11,12] ulcerative colitis,[13,14] and rheumatoid arthritis.[15,16] Given the nature of GWAS studies, namely associations between a SNP and a phenotype, we can only postulate as to the potential role of this locus in the pathogenesis of acute vaso-occlusive pain, a complex phenomenon involving tissue ischemia, hypoxia-reperfusion injury, immune responses and inflammation,[17,18] and interactions between red blood cells, endothelium, and leukocytes regulated by regulated by T-cell cytokines and adhesion molecules.[19,20] Interleukin-2 (IL-2) and interleukin-21 (IL-21) may modulate acute pain in SCD through their effects on inflammation and immune responses. IL-2 is a key cytokine for T-cell activation and proliferation.[21] IL-21 enhances B, T and natural killer cell proliferation and interferon-γ production; inhibiting IL-21 has been shown to dampen inflammatory responses.[22,23] T lymphocytes have also been implicated as mediators of pain hypersensitivity.[24] *KIAA1109* is moderately expressed in all adult and fetal tissues and encodes a protein of unknown function.[25] *TENR* encodes testis nuclear RNA-binding protein, expressed primarily in the testis.

Strengths of the study include the consistent definition of acute severe vaso-occlusive pain requiring hospitalization, the relatively large sample size from two independent cohorts of children with fewer co-morbidities, and lower rates of chronic pain than adults with SCA. Pooling the cohorts improved power for our discovery analysis, but precluded validation in a separate cohort.

In summary, we present preliminary evidence of an association between variant rs3115229 and acute severe vaso-occlusive pain in children with SCA. Our results will require additional validation and functional studies to understand the biology and reveal mechanisms by which candidate SNPs/genes might have their effects.

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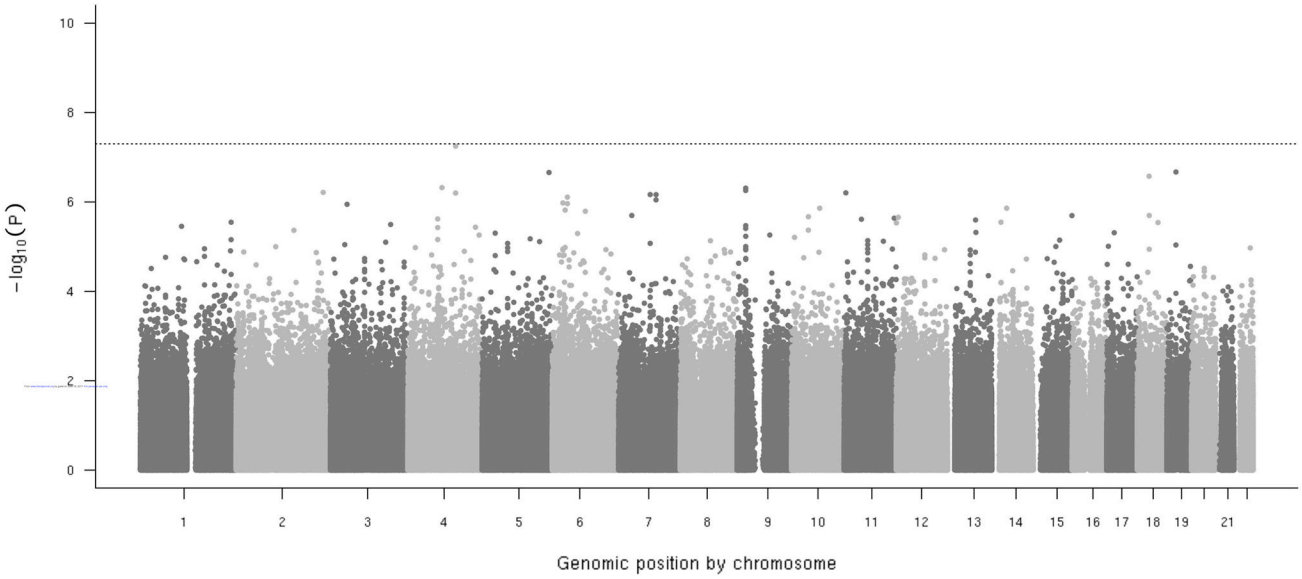

Figure 1. Manhattan plot showing the genome-wide −log$_{10}$ *P* values for association of SNPS with vaso-occlusive pain

Only one SNP on chromosome 4 (rs3115229) approached genome wide significance ($P = 5.63\times10^{-8}$).

Table 1

Summary of SIT and CSSCD cohort demographics and clinical characteristics

Characteristic	CSSCD* (n=349)	SIT trial† (n=934)	P-value‡
Male sex, n (%)	195 (54.3)	489 (52.3)	0.53
Age (in years), mean ± SD	6.92 ± 2.78	8.96 ± 2.44	<0.001
Follow-up (in years), mean ± SD	2.8 ± 0.67	3.00 ± 0.0	<0.001
Acute severe vaso-occlusive pain# rate (events/patient year)	0.58±1.02	0.61 ± 0.83	0.01
ACS rate (events/patient year)	0.18 ± 0.41	0.13 ± 0.26	0.14
Hematocrit (%), mean ± SD	23.10 ± 2.81	23.35 ± 3.43	0.47
Hemoglobin (g/dl), mean ± SD	7.98 ± 0.88	8.12 ± 1.08	0.15
Fetal Hemoglobin (%), mean ± SD	7.59 ± 4.87	8.94 ± 5.75	0.001
Reticulocytes (%), mean ± SD	13.99 ± 5.62	12.02 ± 5.48	<0.001
White Blood Cells, 10^9/L	12.38 ± 2.71	12.58 ± 5.26	0.68

*Age range at enrollment was 2–12 years; pain/ACS events were defined as those occurring 3 years prospectively; therefore, the age range in which they developed pain is 2–15 yrs.

†Age range was 2–15 years at enrollment; pain/ACS events were defined as those occurring 3 years retrospectively; the youngest patient enrolled is 5 years of age

‡P-values for continuous and categorical variables are based on Wilcoxon rank sum test (with continuity correction) and Pearson chi-square test, respectively.

#Acute severe vaso-occlusive pain was defined as an acute episode of pain requiring hospitalization and treatment with opioids.